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(54) Title: COMPOSITIONS AND METHODS FOR HUMAN GASTROINTESTINAL HEALTH

(57) Abstract

A composition for promoting gastrointestinal health comprises an effective amount of a beneficial human intestinal microorganism and an effective amount of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins. Another composition for restoring and maintaining gastrointestinal health comprises 40-60 % by weight of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins and 40-60 % by weight of soluble dietary fiber selected from inulin, fructo-oligosaccharides, pectin, guar gum, and mixtures thereof. The immunoglobulin and fiber-containing composition can optionally contain one or more of a beneficial human intestinal microorganism, components of a non-immune natural defense system, an iron-sequestering molecule, and gluconic acid. Preferred beneficial human intestinal microorganisms include lactobacilli and bifidobacteria. The immunologically active immunoglobulins are preferably purified from bovine milk, milk products, or whey. Methods of use are also described.

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COMPOSITIONS AND METHODS FOR HUMAN GASTROINTESTINAL HEALTH

Background of the Invention

This invention relates to compositions and methods for promoting gastrointestinal health. More particularly, the invention relates to a composition comprising (a) an immunoglobulin preparation containing immunoglobulins that are capable of binding and inactivating foreign antigens such as pathogenic bacteria, viruses, fungi, and protozoa that are detrimental to gastrointestinal health, and (b) living bacteria that are beneficial for gastrointestinal health. The invention also relates to another composition comprising (a) the immunoglobulin preparation containing immunologically-active immunoglobulins; (b) soluble dietary fiber that provides the advantages typically offered by dietary fibers with the additional advantages of not affecting blood glucose or insulin levels, being readily fermented by the intestinal microflora and promoting growth of certain beneficial intestinal microorganisms; and (c) optionally one or more of the following: living intestinal bacteria that are beneficial for gastrointestinal health, lactoperoxidase and/or thiocyanate for strengthening a natural non-immune defense system, lactoferrin for inhibiting detrimental iron-catalyzed processes and harmful microorganisms, and gluconic acid for inhibiting growth of harmful bacteria and stimulating immune function.

Since the time of Hippocrates and throughout the Middle Ages, large doses of whey were prescribed by alchemists for treating many ailments, primarily acute septic conditions. Although it was not then known the reason that whey was useful for treating such conditions, recent studies have shown that whey contains antibodies or immunoglobulins capable of providing passive immunity against various pathogens and their toxic by-products. Antibodies or immunoglobulins are

high molecular weight proteins produced in the bodies of mature animals that enhance immunity to infection by bacteria, viruses, fungi, protozoa, and the like. Antibodies in human and bovine milk promote development of a healthy gastrointestinal tract and provide protection against infections by pathogenic microorganisms. These antibodies interfere with the process that allows such pathogenic microorganisms to adhere to and colonize the intestinal lining. Studies have shown that immunoglobulins from whey are particularly effective against viruses (*e.g.*, rotavirus), bacteria (*e.g.*, *E. coli*, *Vibrio cholerae*, *Salmonella*), fungi (*e.g.*, *Candida*), and protozoa (*e.g.*, *Cryptosporidium*).

Detectable levels of anti-rotavirus antibodies (IgG_1) have been found in raw and pasteurized milk. R.H. Yolken, *Antibody to Human Rotavirus in Cow's Milk*, 312 New Eng. J. Med. 605 (1985). The high temperatures used in processing infant formula, however, destroy all traces of naturally occurring IgG_1. Many infants develop gastroenteritis around 6 months of age, about the time they are weaned from breast milk and started on formula.

Since infants and young children are highly susceptible to gastroenteritis, treatment of acute diarrhea with concentrated immunoglobulins has been investigated. In one study, infants hospitalized with acute rotavirus gastroenteritis were treated with an immunoglobulin concentrate derived from rotavirus-immunized cows. H. Hilpert et al., *Use of Bovine Milk Concentrate containing Antibody to Rotavirus to Treat Rotavirus Gastroenteritis in Infants*, 156 J. Infect. Dis. 158 (1987). These infants showed significantly reduced duration of rotavirus excretion. Thus, bovine milk immunoglobulins provided passive immunity against rotavirus gastroenteritis in human infants.

A bovine milk immunoglobulin concentrate derived from *E. coli*-immunized cows has also been shown to

inhibit colonization of enteropathic *E. coli* in affected infants. C. Mietens et al., *Treatment of Infantile E. Coli Gastroenteritis with Specific Bovine Anti-E. Coli Milk Immunoglobulins*, Eur. J. Pediatrics (1979). Stool samples showed a reduction in *E. coli* counts and the duration of diarrhea was shortened, demonstrating that this concentrate was effective in treating infantile diarrhea.

Inflammation of the gastrointestinal mucosa and diarrhea associated with Traveler's Diarrhea due to *E. coli* infection have been prevented by treatment with an immunoglobulin concentrate from bovine milk. C. Tacket et al., *Protection by Milk Immunoglobulin Concentrate against Oral Challenge with Enterotoxigenic Escherichia Coli*, 318 N. Engl. J. Med. 1240 (1988).

Immunoglobulins from bovine colostrum have been shown to be an effective treatment for diarrhea due to a pathogenic protozoan, *Cryptosporidium*. S. Tzipori et al., *Remission of Diarrhea Due to Cryptosporidiosis in an Immunodeficient Child Treated with Hyperimmune Bovine Colostrum*, 293 Br. Med. J. 1276 (1986). Immunodeficient individuals, particularly those with acquired immune deficiency syndrome (AIDS), are especially susceptible to Cryptosporidiosis.

Certain bacteria have also been shown to be beneficial to human gastrointestinal health. Bacteria of the genus *Lactobacillus* have been used for several hundred years for treating various illnesses. Lactobacilli found in the human intestinal tract include *L. acidophilus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum, and L. cellobiosus*. In recent years, *L. acidophilus* has been shown to be exceptionally useful in treating conditions such as antibiotic-induced imbalances in the gastrointestinal microflora, hypercholesterolemia, vaginal infections, *E. coli* infection, oral contraceptive failure, depressed immunity, cancerous

tumors, chronic granulomatous disease, and lactose indigestion. A.G. Shauss, *Method of Action, Clinical Application, and Toxicity Data*, 3 J. Advancement Med. 163 (1990). *In vitro* studies have shown *L. acidophilus* to have an inhibitory effect on the growth of pathogenic bacteria such as *Campylobacter pylori*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Sarcina lutea*. K.M. Shahani et al., *Natural Antibiotic Activity of Lactobacillus Acidophilus and Bulgaricus*, 11 Cultured Dairy Products J. 14 (1976).

The beneficial effect of *L. acidophilus* is further illustrated by preliminary evidence that *L. acidophilus* inhibits the toxic activities of bacteria in patients with chronic kidney failure. M.L. Simenhoff et al., *Biomodulation of Uremic Pathophysiology in Man*, abstract presented at Am. Soc. of Nephrology Meeting, Baltimore, 1992. Such patients often have toxic levels of amines in their blood due to bacterial overgrowth in the small bowel. Consumption of high levels of freeze dried bacteria drastically reduced levels of these toxic amines. These results demonstrate the ability of *L. acidophilus* to exert a positive effect on the microflora of the intestines.

It has also been shown that the activities of fecal bacterial enzymes thought to play a role in conversion of procarcinogens to carcinogens, such as beta-glucuronidase, nitroreductase, and azoreductase, were reduced 2- to 4-fold in subjects taking *L. acidophilus* supplements. B.R. Goldin & L.S. Gorbach, *The Effect of Milk and Lactobacillus Feeding on Human Intestinal Bacterial Enzyme Activity*, 39 Amer. J. Clin. Nutr. 756 (1984). These results suggest that dietary supplementation with *L. acidophilus* may reduce the risk of developing colon cancer.

Bifidobacteria are also known to exert a beneficial influence on human health. These bacteria exert antimicrobial activity in the human intestine by

producing lactic acid and acetic acid as a result of carbohydrate metabolism. These acids lower the intestinal pH, thereby inhibiting overgrowth of gastrointestinal pathogens. Therapeutic applications of bifidobacteria are indicated for the management of diarrhea and constipation, and the management of hepatic encephalopathy with hyperammonemia. Additional benefits include the production of B vitamins and breakdown of carcinogenic N-nitrosamines.

Bifidobacterium adolescentis is the predominant species of bacteria in humans after age two. This predominance suggests its exceptional stability and prolonged proliferation in the intestine. Nevertheless, for any preparation of living microorganisms to function as a commercial dietary supplement, in addition to being able to provide a beneficial effect must also exhibit good survival in storage, resistance to inactivation by bile, and survival through the gastrointestinal tract. Strain-to-strain or isolate-to-isolate variability can occur as to these traits, thus the selected properties should be verified before commercializing any particular product containing such microorganisms.

Soluble fiber in the diet is also well known for its salutary effects on gastrointestinal health. Such effects include providing bulk to the stool, decreasing the pH of the gastrointestinal tract, producing volatile fatty acids, decreasing intestinal transit time, and beneficially influencing various blood parameters. Dietary fiber has also been shown to have a beneficial effect on cholesterol and lipid metabolism that results in decreased serum cholesterol, triglycerides, and phospholipids and an improved (increased) HDL to LDL ratio. A study on laboratory animals showed that adding fiber to the diet decreases the incidence of bacterial translocation, i.e. crossing the intestinal barrier and entering systemic circulation. C. Palacio et al., *Dietary Fiber: Physiologic Effects and Potential*

Applications to Enteral Nutrition, in Clinical Nutrition: Enteral and Tube Feeding (2d. ed., 1990). Nutritional and epidemiological studies have indicated that a general increase in the consumption of dietary fiber may play a role in preventing deleterious effects of oxygen free radicals that have been accused of being involved in such processes as aging, inflammation, and some disease processes. R. Kohen et al., *Prevention of Oxidative Damage in the Rat Jejunal Mucosa by Pectin*, 69 Br. J. Nutrition 789 (1993).

While prior art formulas as dietary supplements containing soluble dietary fiber or immunoglobulins are known and are generally suitable for their limited purposes, they possess certain inherent deficiencies that detract from their overall utility in restoring and maintaining gastrointestinal health. For example, a dietary supplement containing soluble dietary fiber without concentrated immunoglobulins lacks means for binding and inactivating foreign antigens such as pathogenic bacteria, viruses, fungi, and protozoa that can infect the gastrointestinal tract and are detrimental to the health thereof. Similarly, a dietary supplement containing concentrated immunoglobulins without soluble dietary fiber lacks means for providing bulk to the stool, decreasing the pH of the gastrointestinal tract, producing volatile fatty acids, decreasing intestinal transit time, beneficially influencing various blood parameters, beneficially influencing cholesterol and lipid metabolism, decreasing the incidence of bacterial translocation, preventing deleterious effects of oxygen free radicals, and favoring the growth of beneficial bacteria in the gastrointestinal tract. Further, such prior art formulas fail to provide living intestinal bacteria that are beneficial for gastrointestinal health by providing an inhibitory effect on the growth of pathogenic bacteria, reducing levels of toxic amines, and lowering

the pH of the gastrointestinal tract. Further, prior art dietary supplements fail to provide components, such as lactoperoxidase and thiocyanate, that strengthen the body's natural non-immune defense system or LP-system. Moreover, these formulas do not contain inhibitors of detrimental iron-catalyzed processes and stimulators of immune function.

In view of the foregoing, it will be appreciated that a composition for improving gastrointestinal health comprising living bacteria that exert a beneficial effect on the gastrointestinal tract and an immunoglobulin preparation containing immunoglobulins that bind and inactivate pathogenic microorganisms in the gastrointestinal tract would be a significant advancement in the art. It will also be appreciated that a composition for improving and maintaining gastrointestinal health comprising an immunoglobulin preparation containing immunoglobulins that bind and inactivate pathogenic microorganisms in the gastrointestinal tract and soluble dietary fiber that provides the typical advantages of dietary fiber and additionally is low in calories, does not affect blood glucose or insulin levels, and favors the growth of beneficial bacteria in the gastrointestinal tract while at the same time inhibiting the growth of potentially pathogenic or harmful microorganisms would be another significant advancement in the art.

Objects and Summary of the Invention

It is an object of the present invention to provide a composition for use as a dietary supplement that benefits human gastrointestinal health when administered orally.

It is also an object of the invention to provide a composition for use as a dietary supplement that, when ingested, is effective for treating ailments due to

gastrointestinal pathogens such as bacteria, viruses, fungi, or protozoa.

It is another object of the invention to provide a composition for use as a dietary supplement that, when ingested, results in decreased serum cholesterol, triglycerides, and phospholipids and an increased HDL to LDL ratio.

It is still another object of the invention to provide a composition for use as a dietary supplement that aids in preventing deleterious effects of oxygen free radicals.

It is yet another object of the invention to provide a composition for use as a dietary supplement that bolsters the body's immune system and the natural non-immune system, the LP system.

It is a further object of the invention to provide a composition for use as a dietary supplement that inhibits detrimental iron-catalyzed processes in the body.

These and other objects can be accomplished by providing a composition for use as a dietary supplement for promoting gastrointestinal health comprising an effective amount of a beneficial human intestinal microorganism and an effective amount of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins. Such immunoglobulins can be obtained from any viable source, but are preferably obtained from bovine milk or a milk product. Most preferably, such immunoglobulins are purified from whey. The beneficial human intestinal microorganism is selected from the group consisting of lactobacilli and bifidobacteria. *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* are preferred, and *L. acidophilus* strain NCFM is more preferred. The immunoglobulin composition can further comprise an inert carrier, such as a carbohydrate and/or a lipid.

A method of promoting gastrointestinal health comprises the step of orally administering an effective amount of the bacteria and immunoglobulin-containing composition described above. This method is also effective against bacteria, viruses, fungi, and protozoa that cause diarrhea, constipation, and other forms of gastrointestinal distress.

An immunoglobulin and fiber-containing composition for use as a dietary supplement for restoring and maintaining gastrointestinal health comprises in percent by weight

(a) about 40 to about 60% of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins; and

(b) about 40 to about 60% of soluble dietary fiber, wherein the fiber is a member selected from the group consisting of inulin, fructo-oligosaccharides, pectin, guar gum, and mixtures thereof. The immunoglobulin and fiber-containing composition can optionally contain about 0 to about 20% by weight of a beneficial human intestinal microorganism selected from the group consisting of lactobacilli and bifidobacteria. Preferably, the beneficial human intestinal microorganism is present in an amount in the range of about 0.1 to about 20% by weight, and more preferably of about 5 to about 10% by weight. The immunoglobulin and fiber-containing composition can also optionally contain one or more of the following ingredients:

Ingredient	Ranges in Percent by Weight	
	Broad	Preferred
Lactoperoxidase	0-0.0300%	0.0001-0.0300%
Thiocyanate salt	0-0.0500%	0.0001-0.0500%
Lactoferrin	0-0.1000%	0.0001-0.1000%
Gluconic acid	0-10%	0.1-10%

The beneficial human intestinal microorganism is preferably selected from the group consisting of *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum, L. cellobiosus, Bifidobacterium adolescentis, B. infantis, B. longum, B. thermophilum,* and *B. bifidum.* More preferably, the beneficial human intestinal microorganism is selected from *L. acidophilus* and *B. adolescentis.* A preferred strain of *L. acidophilus* is strain NCFM.

The immunoglobulin composition can also include a carrier. A preferred carrier comprises at least one member selected from the group consisting of a carbohydrate and a lipid, wherein the carbohydrate is capable of being an energy source for a beneficial human intestinal microorganism and the lipid aids in reconstitution of the immunoglobulin composition. A preferred carbohydrate is maltodextrin, and a preferred lipid is lecithin. Preferably, the immunoglobulin composition is purified from a source selected from the group consisting of milk, milk products, and whey, with a bovine source also being preferred.

A method of restoring and maintaining gastrointestinal health comprises the step of orally administering an effective amount of an immunoglobulin and fiber-containing composition for promoting gastrointestinal health comprising in percent by weight

(a) about 40 to about 60% of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins; and

(b) about 40 to about 60% of soluble dietary fiber, wherein the fiber is a member selected from the group consisting of inulin, fructo-oligosaccharides, pectin, guar gum, and mixtures thereof.

Brief Description of the Drawings

FIG. 1 shows growth curves for *Candida* (●) cultured alone, and for a mixed culture of *Candida* (◆) and *L. acidophilus* NCFM (▲).

FIG. 2 shows growth curves for *Candida* (●) cultured alone, and for a mixed culture of *Candida* (◆) and *L. acidophilus* NCFM (▲) also containing an immunoglobulin composition according to the present invention.

FIG. 3 shows growth curves for *Candida* (●) cultured alone, and for *Candida* (◆) cultured in the presence of an equal amount of immunoglobulin composition as in FIG. 2.

FIG. 4 shows growth curves for *S. typhimurium* (●) cultured alone, and for a mixed culture of *S. typhimurium* (◆) and *L. acidophilus* NCFM (▲).

FIG. 5 shows growth curves for *S. typhimurium* (●) cultured alone, and for a mixed culture of *S. typhimurium* (◆) and *L. acidophilus* NCFM (▲) also containing an immunoglobulin composition according to the present invention.

Detailed Description of the Invention

Before the present composition and methods of use are disclosed and described, it is to be understood that

this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a composition containing "a microorganism" includes a mixture of two or more microorganisms, reference to "an immunoglobulin" includes reference to two or more of such immunoglobulins, and reference to "a concentrate" includes reference to a mixture of two or more of such concentrates.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

As used herein, "immunoglobulin composition" means a composition comprising an effective amount of immunologically active immunoglobulins. Preferably, these are present as concentrated immunologically active immunoglobulins. One such immunoglobulin composition is sold under the trademark "PROBIOPLEX" by Metagenics, Inc. (San Clemente, California). PROBIOPLEX contains (1) about 55-60 parts by weight of an immunoglobulin concentrate from bovine whey wherein at least about 7% by weight of the total solids in the concentrate is immunologically active immunoglobulins, (2) about 35-40 parts by weight of a mixture of carbohydrates including rice maltodextrin and lactose, and (3) about 5-10 parts by weight of lipid including lecithin. Thus, at least

about 3.6% by weight of the total PROBIOPLEX composition comprises immunologically active immunoglobulins. The carbohydrates and lipids function as inert carriers for the immunoglobulins. The rice maltodextrin can function further as an energy source for beneficial microorganisms with which the immunoglobulin composition can be mixed in accordance with the present invention. The lecithin aids in dispersion of the powder form of the immunoglobulin composition when reconstituted with water or other liquid. Although PROBIOPLEX contains ingredients other than concentrated immunologically active immunoglobulins, these other ingredients are optional components of the invention. What is required is that the immunoglobulin composition contain an "effective amount" of immunologically active immunoglobulins that are preferably present in concentrated form.

As used herein, "beneficial human intestinal microorganism" means an organism of microscopic size, such as a bacterium, that inhabits the human intestine and exerts a beneficial effect on the gastrointestinal health of an individual in which it resides. Preferred beneficial human intestinal microorganisms according to the present invention include bacteria of the genera *Lactobacillus* and *Bifidobacterium*. A more preferred lactobacillus is *L. acidophilus*, with *L. acidophilus* strain NCFM being most preferred, and a more preferred bifidobacterium is *B. adolescentis*. Other lactobacilli that are beneficial to gastrointestinal health include *L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum, and L. cellobiosus*. Other bifidobacteria that are beneficial to gastrointestinal health include *B. infantis, B. longum, B. thermophilum, and B. bifidum*.

As used herein, "effective amount" means an amount necessary to achieve a selected result. For example, an effective amount of an immunoglobulin and bacteria-

containing composition useful for reducing the titer of a selected pathogenic microorganism in the gastrointestinal tract would be an amount that achieves the selected result of reducing the titer of the microorganism. Such an amount can be readily determined without undue experimentation by a person of ordinary skill in the art. As another example, an effective amount of an immunoglobulin and fiber-containing composition useful for reducing the titer of a selected pathogenic microorganism in the gastrointestinal tract would be an amount that achieves the selected result of reducing the titer of the microorganism. Such an amount can also be readily determined without undue experimentation by a person of ordinary skill in the art.

As used herein, "thiocyanate salt" means a nutritionally acceptable salt of the thiocyanate anion, such as sodium thiocyanate, potassium thiocyanate, ammonium thiocyanate, and mixtures thereof.

As reviewed above, immunoglobulin concentrates from milk contain immunologically active immunoglobulins that are capable of binding pathogenic microorganisms such as bacteria, viruses, fungi, and protozoa. Such immunoglobulin concentrates can be prepared from any starting material containing sufficient concentrations of immunologically active immunoglobulins, such as milk, whey, blood, and the like. An economically viable source of such immunoglobulins is the whey byproduct of the cheese making process. It has been estimated that approximately 85 million metric tons of whey are created annually as a byproduct of cheese production worldwide. About 34 million metric tons of whey are not economically utilized, and thus are discarded. The whey byproduct of cheese making, therefore, presents an inexpensive and ready source of immunoglobulins.

Numerous techniques are known to exist for producing dry concentrated protein extract from whey.

This protein extract is commonly referred to as whey protein concentrate or "WPC." Such protein extraction and concentration techniques have been primarily concerned with preserving the food qualities of the WPC, such as taste, flavor, and solubility. Although these techniques are useful for producing food products, they almost universally destroy or substantially reduce the immunological activity of immunoglobulins in the concentrate by exposing the raw milk, whey, or protein concentrate to (1) excessive thermal (time and temperature) conditions, (2) excessive bacterial activity, or (3) excessive enzymes added in processing or resulting from bacterial activity.

Methods have been developed for separating immunologically active immunoglobulins from raw milk. U.S. Patent Nos. 4,816,252 and 4,834,974 describe such methods, which are illustrative of methods that can be used for preparing an immunologically active immunoglobulin concentrate according to the present invention. Raw milk is first flash pasteurized to control microbial activity in the milk without significantly diminishing the immunological activity of the immunoglobulins in the milk. Next, the milk is exposed to an appropriate cheese starter culture, such as a lactobacillus, at carefully controlled temperatures and for limited times to achieve a selected degree of curd formation without significantly affecting the immunological activity of the immunoglobulins. The whey is then separated from the cheese curd and transferred to a clarifier or separator under carefully controlled conditions to remove fat and casein particles. The clarified whey is then subjected to ultrafiltration to remove or substantially reduce the amounts of small proteins, salts, and other non-protein materials in the retained protein concentrate or retentate. Ultrafiltration can be performed in stages to optimize purification of the immunoglobulins. Optionally, other

concentration and purification steps, such as reverse osmosis and ion exchange chromatography, can then be used to further improve the purity and concentration of the immunoglobulin concentrate while maintaining the immunological activity thereof. The immunoglobulin concentrate is then dried through conventional freeze-drying or spray drying methods. The resulting dry immunoglobulin concentrate can then be stored at room temperature. At least about 7% of the total solids of immunoglobulin concentrates prepared by these methods comprise immunologically active immunoglobulins. When ultrafiltration and ion exchange chromatography are both used in the purification procedure, the proportion of immunologically active immunoglobulins as a percentage of total solids can be increased to at least about 50%. Repeated ion exchange chromatography steps can further increase the proportion of immunologically active immunoglobulins as a percentage of total solids. U.S. Patent Nos. 4,816,252 and 4,834,974 are hereby incorporated herein by reference as illustrative of methods for purifying immunologically active immunoglobulin concentrate. The present invention is not limited to these methods, however, and any method of purifying and concentrating immunologically active immunoglobulins from milk, whey, or another suitable source is to be considered within the scope of the invention as long as an effective amount of immunologically active immunoglobulins is obtained in the "immunoglobulin composition." Bovine milk and bovine whey are preferred sources of immunoglobulins, but other species of animal could also be used.

Certain bacteria have also been shown to be beneficial to human gastrointestinal health, as briefly reviewed above. The intestinal flora of the human gut contains some 100 x 10^9 viable bacteria, representing 100 or more different species. The major bacteria can be roughly divided into three groups: (a) lactic acid

bacteria, including lactobacilli, bifidobacteria, and streptococci; (b) anaerobic bacteria; and (c) aerobic bacteria.

Bacteria of the genus *Lactobacillus* have been used for several hundred years for treating various illnesses. Bifidobacteria are also known to exert a beneficial influence on human health. Bifidobacteria constitute the predominant microorganisms in the fecal flora of week-old breast-fed infants, making up 85-99% of the bacterial population. Upon weaning or upon the occurrence of perturbations such an infection, vaccination, a sudden change in diet, and even the weather the balance of microorganisms in the gastrointestinal tract of these babies can be upset. Bifidobacteria can also be significantly reduced in elderly people due to a reduction of secreted gastric juices. The bifidobacterial population in adults is much more stable, however changes in diet, administration of antibiotics, exposure to gamma radiation or X-rays, disease, stress, and other disturbances can result in overgrowth of potentially pathogenic bacteria, decrease in beneficial bacteria (lactobacilli and bifidobacteria), and a resulting imbalance in the gastrointestinal flora. Hyperproliferation of harmful bacteria in the gut is associated with various forms of diarrhea, susceptibility to systemic infections, constipation, vague and acute abdominal symptoms, fatigue, dyspepsia, and presence of carcinogenic metabolites. Reestablishment of a normal balance of gastrointestinal flora can be accelerated, and such normal balance maintained, with dietary administration of lactobacilli and/or bifidobacteria.

Lactobacilli and bifidobacteria produce organic acids that reduce intestinal pH and thereby inhibit the growth of acid-sensitive undesirable bacteria. Lactobacilli produce lactic acid, hydrogen peroxide, and

possibly acetic and benzoic acids. Bifidobacteria produce short chain fatty acids (SCFA) such as acetic, propionic, and butyric acids, as well as lactic and formic acids. The most plentiful short chain fatty acid produced by bifidobacteria is acetic acid, which has a wide range of antimicrobial activities against yeasts, molds, and other bacteria. Additionally, short chain fatty acids support normal gastrointestinal function by increasing colonic blood flow, stimulating pancreatic enzyme secretion, promoting sodium and water absorption, and potentiating intestinal mucosal growth. Bifidobacteria are also known to deconjugate bile salts to free bile acids, which are more inhibitory to susceptible bacteria than are the conjugated forms. Further, lactobacilli and bifidobacteria are able to produce other antimicrobial substances, such as bacteriocins, that inhibit the growth and proliferation of harmful bacteria in the gut.

The advantages of soluble dietary fiber have also been briefly reviewed above. Inulin is one such fiber that is composed of a mixture of oligomers and polymers of fructose. Inulin is a storage carbohydrate found in many plants including onion, asparagus, artichoke, and many cereals. Chicory root and Jerusalem artichoke each contain about 70% by weight of inulin. Inulin has been an important food in Europe for many years and is currently being used as a source of dietary fiber, for replacing fat in the diet, and for promoting growth of beneficial bacteria in the intestine. In the U.S., inulin is added to all types of noodles. It has a moderately sweet taste, is highly soluble, and is a frequent replacement for sugar in many foods. Medically, inulin is the substance of choice to study renal clearance and impaired kidney function.

Fructo-oligosaccharides (FOS) are another type of soluble dietary fiber. FOS is widely distributed in nature and is found in honey, beer, onion, asparagus,

Chinese chive, banana, maple sugar, oats, and Jerusalem artichoke.

Upon ingestion, both inulin and FOS are hydrolyzed to a negligible extent as they pass through the mouth, stomach, and small intestine. In the large intestine, they are readily fermented by the intestinal microflora. These carbohydrates are metabolized by the bacteria into short chain fatty acids, mainly acetic, propionic, butyric, and lactic acids. As a consequence of this fermentation, a considerable amount of bacterial mass is produced, which increases stool wet weight. The short chain fatty acids are absorbed by the large intestine and are further metabolized in the liver. This allows the body to recover some energy from inulin and FOS, although the efficiency of energy conversion is markedly lower than with other carbohydrates. This phenomenon underlies the low calorie content of fructans and dietary fibers.

Inulin and FOS are used as a source of energy in the intestinal tract mainly by bacteria in the genus *Bifidobacterium*. H. Hidaka et al., *Effects of Fructooligosaccharides on Intestinal Flora and Human Health*, 5 Bifidobacteria Microflora 37-50 (1986). When inulin and FOS are administered in the diet, the bifidobacteria increase significantly, becoming the predominant bacteria in the intestinal population, and the clostridia, which are a measure of potentially pathogenic microorganisms, are significantly reduced. As will be discussed in more detail below, bifidobacteria are human intestinal bacteria that provide beneficial effects on gastrointestinal health. Other important groups of bacteria in the mixed population in the intestines, such as *Fusobacterium*, *Lactobacillus*, and aerobic bacteria, are not significantly affected by the administration of inulin and FOS. H. Hidaka et al., *Effects of*

Fructooligosaccharides on Intestinal Flora and Human Health, 5 Bifidobacteria Microflora 37-50 (1986).

It has been shown, A. Hata, *The Influence of Neosugar on the Lipid Metabolism of Experimental Animals*, Proc. 1st Neosugar Res. Conference, Tokyo (1982), that fructo-oligosaccharides (FOS) in the diet of experimental animals cause reduction of blood sugar, serum cholesterol, triglycerides, and phospholipids; significant improvement in the HDL/LDL ratio; an increase in free fatty acids; and significant decreases in total cholesterol in lipedemia cases.

It has also been shown, H. Hadaka et al., *Effects of Fructooligosaccharides on Intestinal Flora and Human Health*, 5 Bifidobacteria Microflora 37-50 (1986), that administration of fructo-oligosaccharides (FOS) enhances growth of the bifidobacteria population in the intestine, suppresses production of putrefactive factors, improves blood lipid levels in hyperlipidemia patients, and provides relief from constipation.

Therefore, at least the following positive effects are obtained by addition of inulin and/or fructo-oligosaccharides (FOS) to a composition for use as a dietary supplement according to the present invention: reduction of intestinal disorders, enhancement of a balanced intestinal microflora, and remediation of constipation.

Other preferred dietary fibers according to the present invention include pectin and guar gum. Pectin is a highly water soluble, noncellulosic polysaccharide fiber extracted from the primary cell walls of plants. Rich sources of pectin include lemon and orange rinds, which contain about 30% by weight of this polysaccharide. Pectin occurs naturally as a partial methyl ester of α-(1→4) linked D-polygalacturonate sequences interrupted with (1→2)-L-rhamnose residues. Pectins are used as gelling and thickening agents in food technology and as an antidiarrheal in veterinary

medicine. Guar gum is produced from the ground endosperms of *Cyamopsis tetragonolobus*, a legume cultivated in India as a livestock feed. The water soluble fraction, which comprises about 85% of guar gum and is known as guaran, consists of linear chains of (1→4)-ß-D-mannopyranosyl units with α-D-galactopyranosyl units attached by (1→6) linkages. The ratio of D-galactose to D-mannose is 1:2. Guar gum has 5 to 8 times the thickening power of starch and, thus, is used as a thickener in foods, as a binder and disintegrating agent in tablet formulations, and in pharmaceuticals and cosmetics.

Pectin and guar gum have several beneficial effects on the gastrointestinal tract, such as maintaining the morphology of intestinal villi, increasing lipase activity in the small bowel, delaying gastric emptying time, increasing intestinal transit time, and promoting increased fecal production of short chain fatty acids. It is believed that pectin and guar gum in the diet lower blood glucose and serum cholesterol levels, B. Flourie et al., *The Effect of Pectin on Jejunal Glucose Absorption and Unstirred Layer Thickness in Normal Man*, 25 Gut 1936 (1984). Also, dietary fiber supplementation with pectin or guar gum has also been found to significantly suppress the incidence of colon cancer. G. Arbman, *Cereal Fiber, Calcium and Colorectal Cancer*, 69 Cancer 2042 (1992). Studies with whole apples show that fiber (pectin) in the fruit reduces the insulin response to the sugar in the fruit and prevents "rebound" hypoglycemia. D. Jenkins et al., *Dietary Fiber, Fiber Analogues and Glucose Tolerance, Importance of Viscosity*, 1 Br. Med. J. 1392 (1978). Further, pectin and guar gum are readily degraded by bacterial fermentation in the colon, probably because of their high water solubility.

Moreover, pectin and guar gum are also thought to prevent oxidative damage in the gastrointestinal tract.

Oxygen free radicals are involved in many deleterious processes including aging, inflammation, and some disease processes. The gastrointestinal mucosa is exposed to oxidants produced within the lumen and in the epithelial cells. Potential sources of luminal oxidants include ingested food, catalase-negative bacteria, and cigarette smoke and other pollutants. The production of reactive free radicals during metabolism of dietary fat can explain some the biological damage such as loss of membrane function, inactivation of membrane-bound enzymes, and inactivation of essential molecules located inside the cell. Other tests have shown that a large amount of fat in the diet can be a presumptive carcinogen. H. Hidaka et al., *Effects of Fructooligosaccharides on Intestinal Flora and Human Health*, 5 Bifidobacteria Microflora 37-50 (1986). Apart from these carcinogenic changes, still other injuries associated with free radicals include ulcerative diseases, inflammation, and ischemic bowel disease. Pectin and guar gum prevent oxidative damage in various ways. They directly scavenge intestinal oxidants. Further, pectin can act as a chelating agent of loosely bound transition metals in the lumen. Moreover, pectin also reacts directly to prevent spontaneous dismutation of superoxide radicals and thus prevents the formation of hydrogen peroxide.

In human and animal tissues, peroxidases form part of a natural non-immune defense system and also play a role in protecting against microbial invasion of mucous membranes. Peroxidases occur in various exocrine gland secretions including salivary, lachrymal, bronchial, nasal, and intestinal secretions and in milk. Milk peroxidases, known as lactoperoxidases (LP) are the predominant enzymes in bovine milk. LP has no intrinsic antibacterial activity, however, together with hydrogen peroxide and thiocyanate anion it forms a potent natural antibacterial system, the so-called lactoperoxidase or

LP system (for review see B. Reiter, *Bacterial Inhibitors in Milk and Other Secretions with Special Reference to the Complement, Transferrin and Lactoperoxidase/Thiocyanate/Hydrogen Peroxide Systems, in* Inhibition and Inactivation of Vegetative Microbes 31-60 (1976); B. Reiter & J.-P. Perraudin, *Lactoperoxidase: Biological Functions, in* 1 Peroxidases in Chemistry and Biology 143-180 (1991). The antibacterial effect of the LP system is mediated by the generation of short-lived oxidation products of thiocyanate anion (SCN), mainly the hypothiocyanate ion (OSCN). LP is a highly active enzyme, and very low concentrations are sufficient to establish an effective system. A wide range of bacterial species is affected by the LP system. Gram-negative bacteria generally are killed or their growth inhibited. Gram-positive bacteria usually are more resistant, however, and in general only their growth is inhibited. The LP system can also affect certain viruses, yeasts, and molds.

The thiocyanate anion is widely distributed in animal and human tissues, body-fluids, and secretions. It is found in the mammary, salivary, and thyroid glands, in the stomach and kidneys, in synovial, cerebral, and spinal fluid, and in lymph and plasma. The major dietary sources of thiocyanate ion are vegetables such as cabbage, cauliflower, and turnip, which are rich in glucosinolates that yield thiocyanate ion upon hydrolysis.

The activity of the LP system arises from an LP-catalyzed reaction in which hydrogen peroxide oxidizes thiocyanate ion (SCN) to form the hypothiocyanate ion (OSCN). The hypothiocyanate ion then oxidizes sulfhydryl groups in vital metabolic enzymes and other proteins of the microorganisms. The mechanisms of antimicrobial activity of the LP system result in damage to bacterial membranes and inhibition of essential transport mechanisms, such as those involving glucose

and amino acids, and inhibition of synthesis of nucleic acids and proteins, including vital metabolic enzymes such as those involved in glycolysis.

Microorganisms inhibited by the LP system include a number of Gram-positive bacteria, including species of *Staphylococcus* and *Streptococcus*, and some Gram-negative species, *e.g.*, *E. coli*, *Salmonella*, *Pseudomonas*. Some lactic acid bacteria, *e.g.* lactobacilli and bifidobacteria, are unaffected by the LP system because they contain a "reversal enzyme" called NAD(P)-OSCN-oxidase reductase, which prevents the antimicrobial activity of the LP system.

Lactoperoxidase is a highly active enzyme, and very low concentrations, along with low concentrations of hydrogen peroxide and thiocyanate ion, are sufficient to obtain an effective system. Hydrogen peroxide is known to be produced in many species of lactobacilli, and thiocyanate ion is widely distributed in animal and human tissues, body fluids, and secretions.

Advantages of the LP system include a greater antimicrobial efficacy and a wider spectrum of activity than existing preservatives. Also, the active antimicrobial agents of the LP system (OSCN and HOSCN) disappear from food after processing, thus providing a safe, long-lasting food preservative without the presence of the active preservative agents. Further, the LP system acts in synergy with other preservatives, thus increasing the efficacy of such other preservatives. Moreover, the LP system has a very low level of toxicity.

Lactoferrin is an iron-binding protein present in milk. For example, bovine milk contains about 200 mg/l of lactoferrin, and human milk and colostrum contain about 2-4 g/l and 6-8 g/l of lactoferrin, respectively. The affinity of lactoferrin for iron is very high, e.g. about 300 times that of the iron-transporting protein, transferrin, in blood plasma. A lactoferrin molecule

binds one ferric ion (Fe^{3+}) by means of a bicarbonate-dependent reaction.

The high affinity for iron enables the use of lactoferrin for inhibiting iron-catalyzed processes, such as generation of free hydroxyl radicals, lipid peroxidation, and growth of microorganisms. Most microorganisms need iron for growth. Lactoferrin is able to inhibit the growth of such microorganisms by depriving them of iron. Lactoferrin is bacteriostatic to a wide range of microorganisms, including Gram-negative bacteria with a high iron requirement and some Gram-positive bacteria. Lactic acid bacteria, such as lactobacilli and bifidobacteria, have a low iron requirement and, in general, are not affected by lactoferrin. Although lactoferrin is primarily bacteriostatic, heat-treated lactoferrin is bactericidal. Heat-treated lactoferrin is easily obtained by heating lactoferrin at acidic pH.

Lactoferrin has been demonstrated in *in vitro* and *in vivo* tests to be effective against a variety of microorganisms, including *E. coli*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Candida albicans*, while at the same time promoting the growth of bifidobacteria. Lactoferrin retains iron at low pH and can pass through the acid environment of the stomach and enter the intestine unaltered.

As described above, various indigestible saccharides, such as FOS, have been developed for promoting the growth of bifidobacteria. Another substance that promotes the growth of bifidobacteria is gluconic acid and its salts (gluconates). It has been shown in *in vitro* fermentation tests that gluconate is utilized selectively by bifidobacteria as an energy source. H. Sato et al., *Antioxidant Activity of Synovial Fluid, Hyaluronic Acid, and Two Subcomponents of Hyaluronic Acid*, 31 Arthritis & Rheumatism (1988). In addition to promoting the growth of bifidobacteria,

gluconic acid, like other organic acids, also suppresses the growth of certain harmful bacteria, such as *Clostridium perfringens*. Test results have further shown that ingested gluconic acid and gluconates are not absorbed in the small intestine, but instead are able to reach the large intestine where they can be utilized as an energy source by bifidobacteria. Sato et al., *Antioxidant Activity of Synovial Fluid, Hyaluronic Acid, and Two Subcomponents of Hyaluronic Acid*, 31 Arthritis & Rheumatism (1988).

Bacteria and Immunoglobulin-Containing Composition

A bacteria and immunoglobulin-containing composition according to the present invention comprises a mixture of an immunoglobulin composition and a beneficial human intestinal bacterium, such as a lactobacillus or a bifidobacterium or mixtures thereof. The composition is made by mixing dry immunologically active immunoglobulins with dry beneficial human intestinal bacteria. The bacteria are prepared, for example, by culturing in a rich medium such as LB, J. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1972), until the late log phase of growth is reached. The bacteria are then concentrated and lyophilized according to standard methods. The dry immunoglobulins and dry bacteria are then mixed in selected proportions. Just prior to consumption, the dry composition is reconstituted with water, juice, or the like to result in a smooth liquid composition that can be consumed orally.

It has been found that oral administration of such a bacteria and immunoglobulin-containing composition has a beneficial effect on gastrointestinal health. Although immunoglobulin compositions containing immunologically active immunoglobulins and beneficial bacteria such as lactobacilli and bifidobacteria each

have some effect on diminishing the growth of pathogenic microorganisms in the gastrointestinal tract, it has been surprising to discover that a composition containing a mixture of the immunoglobulin composition and beneficial bacteria has a synergistic effect in causing death of the pathogenic microorganisms and in restoring gastrointestinal health. Regular consumption of the bacteria and immunoglobulin-containing composition has the effect of maintaining good gastrointestinal health. The bacteria and immunoglobulin-containing composition contains an effective amount of each of the bacterial and immunoglobulin components, and preferably contains weight ratios of bacteria to immunologically active immunoglobulins in the range of about 20:1 to about 1:20. More preferably, the weight ratios of bacteria to immunologically active immunoglobulins are in the range of about 1:5 to about 10:1.

The effects of exposing pathogenic microorganisms to bacteria and immunoglobulin-containing compositions according to the present invention are illustrated in the following examples. These examples are merely illustrative and are not intended to delimit the scope of the invention.

EXAMPLE 1

In vitro cultures of *Candida albicans* were prepared by subculturing from a stock culture in a rich liquid medium. Cultures were incubated at 37°C, and cells were counted by dilution and plating on plate count agar. FIG. 1 shows cell viability in cultures containing *C. albicans* alone (●) and cultures containing both *C. albicans* (◆) plus *L. acidophilus* strain NCFM (▲). During the course of this study, the *C. albicans* multiplied at the same rate regardless of the presence or absence of the *L. acidophilus* NCFM. The number of viable *L. acidophilus* NCFM cells, however, was

diminished by a factor of about 20 in the presence of *C. albicans* cells.

EXAMPLE 2

FIG. 2 shows the cell viability in cultures containing *C. albicans* alone (●) and cultures containing both *C. albicans* (♦) and *L. acidophilus* strain NCFM (▲) as in Example 1, with the exception that the immunoglobulin composition containing immunologically active immunoglobulins was added to the mixed cultures of *C. albicans* plus *L. acidophilus* strain NCFM in a weight ratio of 1 part of *L. acidophilus* strain NCFM to 5 parts of immunoglobulin composition. Two predominant differences occurred in this example compared to Example 1. First, the viability of *L. acidophilus* strain NCFM was enhanced by a factor of about 4 to 5 in the presence of the immunoglobulin composition as compared to cultures in which the immunoglobulin composition was absent. Second, the viability of *C. albicans* was greatly reduced after about 20 hours of co-culturing with *L. acidophilus* strain NCFM in the presence of the immunoglobulin composition. In other experiments, it has been found that the immunoglobulin composition by itself did not affect the viability of *C. albicans* (FIG. 3). Thus, although neither *L. acidophilus* strain NCFM nor the immunoglobulin composition alone affected the growth and viability of *C. albicans in vitro*, the mixture of *L. acidophilus* strain NCFM and the immunoglobulin composition caused a rapid decline in the viability of *C. albicans*. Further, the growth and viability of *L. acidophilus* strain NCFM was enhanced in co-culture with *C. albicans* in the presence of the immunoglobulin composition as compared to when the immunoglobulin composition was absent. These results were unforeseen, i.e. that the combination of beneficial bacteria and immunoglobulins would yield a better result than the additive effects of the bacteria and the

immunoglobulins, and that the immunoglobulins would improve the viability of the bacteria in co-culture with another microorganism. Further, these results were considered predictive of what would occur *in vivo* since lactobacilli are known to survive in the gastrointestinal tract and immunoglobulins have been shown to provide passive immunity to certain pathogens upon oral administration.

EXAMPLE 3

In vitro cultures of *Salmonella typhimurium* were prepared by subculturing from a stock culture in a rich liquid medium, J. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1972). Cultures were incubated at 37°C, and cells were counted by dilution and plating on plate count agar.

FIG. 4 shows growth curves for cultures containing *S. typhimurium* alone and cultures containing *S. typhimurium* plus *L. acidophilus*. Cultures containing *S. typhimurium* (●) alone reached stationary phase with a maximum number of viable cells after about 10 hours of growth. Cultures containing a mixture of *S. typhimurium* and *L. acidophilus* strain NCFM also resulted in maximum numbers of viable cells of *S. typhimurium* (◆) at about 10 hours, although the number of viable cells was diminished about 100-fold compared to *S. typhimurium* cultured alone. The cell viability of *L. acidophilus* strain NCFM (▲) appeared to unaffected by the presence of *S. typhimurium*.

EXAMPLE 4

FIG. 5 shows the cell viability in cultures containing *S. typhimurium* alone (●) and cultures containing both *S. typhimurium* (◆) plus *L. acidophilus* strain NCFM (▲) as in Example 4 with the exception that the immunoglobulin composition containing

immunologically active immunoglobulins was added to the mixed cultures of *Candida* plus *L. acidophilus* strain NCFM in a weight ratio of 1 part of *L. acidophilus* strain NCFM to 5 parts of immunoglobulin composition. These results show that when *S. typhimurium* is cultured in the presence of both *L. acidophilus* strain NCFM and whey immunoglobulins, the *S. typhimurium* failed to produce as many viable cells after 10 hours of growth, and the viability of *S. typhimurium* was greatly reduced through the duration of the experiment as compared to growth in co-culture with *L. acidophilus* strain NCFM without the immunoglobulins. Therefore, the mixture of *L. acidophilus* strain NCFM and the immunoglobulin composition greatly decreased the viability of *S. typhimurium in vitro* compared to growth in the presence of either the immunoglobulin composition or *L. acidophilus* strain NCFM alone. There appears to be an unexpected synergistic effect in diminishing *S. typhimurium* viability by combining the immunoglobulin composition and *L. acidophilus*.

EXAMPLE 5

A strain of *E. coli* isolated from human intestine was cultured alone, in the presence of *L. acidophilus* strain NCFM, and in the presence of both *L. acidophilus* strain NCFM and the immunoglobulin composition in a weight ratio of about 1:10. The results were similar to those of Examples 4 and 5, wherein the viability of the *E. coli* was greatly diminished in the presence of both *L. acidophilus* strain NCFM and the immunoglobulin composition as compared to in the presence of either alone.

The composition of the present invention can be used for maintaining gastrointestinal health as well as for treating diarrhea, constipation, and other types of gastrointestinal distress due to infection with pathogenic microorganisms such as *E. coli, Salmonella,*

Candida, rotavirus, and *Cryptosporidium* by orally administering an effective amount of the composition. The effective amount will vary depending on the size and age of the individual, whether the selected effect is to maintain gastrointestinal health or to restore gastrointestinal health from distress due to infection with a pathogenic microorganism, the particular pathogenic microorganism involved, and the like. A person skilled in the art can routinely determine such an effective amount. The dry ingredients of the composition are stirred into water or juice, and the resulting suspension is taken by mouth. Preferably, dosage is in the range of about 1 to about 100 mg/kg of body weight. More preferably, dosage is in the range of about 5 to about 50 mg/kg of body weight. Doses of the bacteria and immunoglobulin-containing composition can be divided, wherein two or more administrations of divided doses are used to deliver a complete dose. Multiple doses can also be administered, but it is recommended that daily consumption be limited to 1 to 3 doses.

EXAMPLE 6

An adult afflicted with diarrhea due to infection with *Salmonella* was treated with a composition according the present invention containing about 5 parts by weight of *L. acidophilus* NCFM and about 1 part by weight of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins purified from bovine whey. Doses of about 10 mg/kg of body weight were taken by mouth 3 times daily by stirring into water or juice and drinking the resulting suspension. Symptoms began to subside within 24 hours and had completely disappeared within 3 days.

EXAMPLE 7

A small child afflicted with diarrhea due to rotavirus infection was treated with a composition according the present invention containing 5 parts by weight of *B. adolescentis* and 1 part by weight of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins purified from bovine whey. A dose of about 20 mg/kg of body weight was taken by mouth once daily by stirring into water or juice and drinking the resulting suspension. Symptoms began to subside within 24 hours and had completely disappeared within 3 days.

EXAMPLE 8

An adult afflicted with diarrhea due to infection with *Cryptosporidium* is treated with a composition according the present invention containing a weight ratio of about 5:1 of *L. acidophilus* NCFM to concentrated immunologically active immunoglobulins purified from bovine whey. Doses of about 10 mg/kg of body weight are taken by mouth 3 times daily by stirring into water or juice and drinking the resulting suspension. Good gastrointestinal health is restored.

EXAMPLE 9

An adult afflicted with diarrhea due to infection with *Candida* is treated with a composition according to the present invention containing a weight ratio of about 1:5 of *B. adolescentis* to concentrated immunologically active immunoglobulins purified from bovine whey. Doses of about 5 mg/kg of body weight are taken by mouth 3 times daily by stirring into water or juice and drinking the resulting suspension. Good gastrointestinal health is restored.

EXAMPLE 10

An adult who averages 10 episodes of gastrointestinal distress per year takes a daily dose of about 50 mg/kg of body weight of a 5:1 weight ratio of the bacteria and immunoglobulin-containing composition according to the present invention with water or juice. In the ensuing year, only 1 episode of gastrointestinal distress is experienced. This example shows that not only can the bacteria and immunoglobulin-containing composition of the present invention be used for treating acute cases of gastrointestinal distress, but is also effective as a dietary supplement in maintaining good gastrointestinal health.

EXAMPLE 11

Various formulations of the bacteria and immunoglobulin-containing composition are tested in treating acute episodes of gastrointestinal distress, as summarized in Table 1.

Table 1

Bacteria[a]	Immunoglobulins[a]	Condition	Result[b]
0.2	1	diarrhea	+++
0.2	25	diarrhea	+
5	1	diarrhea	+
5	25	diarrhea	+++
0.1	100	diarrhea	-
100	0.1	diarrhea	-
0.5	2.5	diarrhea	+++
0.5	10	diarrhea	+++
2	2.5	diarrhea	+++
2	10	diarrhea	+++
1	4	constipation	+++
4	1	constipation	++
1	3	gas/cramps	+++
3	1	gas/cramps	++

a Parts by weight.
b Symbols represent a relative scale for restoring gastrointestinal health: +++, excellent; ++, very good; +, good; -, poor.

Immunoglobulin and Fiber-Containing Composition

In accordance with a preferred embodiment of the present invention, there is provided an immunoglobulin and fiber-containing composition for use as a dietary supplement. The formulation preferably includes a mixture of an immunoglobulin composition and a soluble dietary fiber selected from the group consisting of inulin, fructo-oligosaccharide, pectin, guar gum, and mixtures thereof in optimal ratios to restore and maintain good gastrointestinal health.

In its most fundamental form, the immunoglobulin and fiber-containing formulations of the present invention include a mixture of about 40 to about 60% by weight of an immunoglobulin composition comprising

concentrated immunologically active immunoglobulins and about 40 to about 60% by weight of soluble dietary fiber selected from the group consisting of inulin, fructo-oligosaccharides, pectin, guar gum, and mixtures thereof.

It is also preferable that the formulation contain a beneficial human intestinal microorganism for restoring and maintaining good gastrointestinal health. The beneficial human intestinal microorganism is preferably a member selected from the group consisting of lactobacilli and bifidobacteria. Preferred lactobacilli include *L. acidophilus, L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum,* and *L. cellobiosus. L. acidophilus* is more preferred and *L. acidophilus* strain NCFM is most preferred. Preferred bifidobacteria include *B. adolescentis, B. infantis, B. longum, B. thermophilum,* and *B. bifidum. B. adolescentis* is more preferred. Such beneficial human intestinal bacteria can be added to the base formulation in an amount in the range of about 0 to about 20% by weight, preferably about 0.1 to about 20% by weight, and more preferably about 5 to about 10% by weight.

It is also preferable that the formulation contain one or more additives for enhancing the activity of the body's non-immune defense system known as the LP system. Such additives can be added to the base formulation, with or without the presence of optional ingredients, in the following concentrations: lactoperoxidase in an amount in the range of about 0 to about 0.0300% by weight and thiocyanate salt in an amount in the range of about 0 to about 0.0500% by weight. Preferably, lactoperoxidase is present in an amount in the range of about 0.0001 to about 0.0300% by weight, and thiocyanate salt is present in an amount in the range of about 0.0001 to about 0.0500% by weight.

It is also preferable that the formulation contain additional optional ingredients for inhibiting the growth of harmful intestinal microorganisms and/or promoting the growth of beneficial human intestinal microorganisms, such as bifidobacteria. Such additives can be added to the base formulation, with or without the presence of other optional ingredients, in the following concentrations: lactoferrin in an amount in the range of about 0 to about 0.1000% by weight and gluconic acid, its nutritionally acceptable salts, or mixtures thereof in an amount in the range of about 0 to about 10% by weight. Preferably, lactoferrin is present in an amount in the range of about 0.0001 to about 0.1000% by weight, and gluconic acid, its nutritionally acceptable salts, or mixtures thereof in an amount in the range of about 0.1 to about 10% by weight.

The composition is preferably manufactured in powder form by agglomerating the dry, raw material ingredients in a suitable agglomerator so as to result in a finished product having a uniform composition with the precise proportions of the components. The bacteria are prepared, for example, by culturing in a rich medium such as LB, J. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1972), until the late log phase of growth is reached. The bacteria are then concentrated and lyophilized according to standard methods. The agglomerated material is then packaged in a suitable container. Just prior to consumption, the dry composition is reconstituted with water, juice, or the like to result in a smooth liquid composition that can be consumed orally. If desired, the composition can be formulated in liquid form. The preferred daily dosage of the formula ranges from about 5 to about 15 grams based on the powdered composition. The daily dosage can be ingested in a single serving or divided into various servings and taken at intervals. Preferably, the

composition of the present invention is taken between meals.

The composition of the present invention can be used for maintaining gastrointestinal health as well as for treating diarrhea, constipation, and other types of gastrointestinal distress due to infection with pathogenic microorganisms such as *E. coli*, *Salmonella*, *Candida*, rotavirus, and *Cryptosporidium* by orally administering an effective amount of the composition. The effective amount will vary depending on the size and age of the individual, whether the selected effect is to maintain gastrointestinal health or to restore gastrointestinal health from distress due to infection with a pathogenic microorganism, the particular pathogenic microorganism involved, and the like. A person skilled in the art can routinely determine such an effective amount. The dry ingredients of the composition are stirred into water or juice, and the resulting suspension is taken by mouth. Preferably, dosage is in the range of about 20 to about 400 mg/kg of body weight. More preferably, dosage is in the range of about 70 to about 215 mg/kg of body weight. Doses of the bacteria and immunoglobulin-containing composition can be divided, wherein two or more administrations of divided doses are used to deliver a complete dose. Multiple doses can also be administered, but it is recommended that daily consumption be limited to 1 to 3 doses.

EXAMPLE 12

The following formulas represent specific embodiments of the invention. These may be prepared in the manner indicated above by blending together the stated raw ingredients in an agglomerator so as to result in a finished product having uniform composition with the precise proportions of the components as indicated. The agglomerated material is then packaged in a suitable container. In the preferred embodiment,

the formula comprises the following ingredients stated in amounts by weight:

Formulation A

Inulin	50%
Immunoglobulin comp.	50%

Formulation B

Inulin	40%
Immunoglobulin comp.	40%
L. acidophilus NCFM	20%

Formulation C

Pectin	40%
Immunoglobulin comp.	60%

Formulation D

Guar Gum	20%
Pectin	30%
Immunoglobulin comp.	40%
B. adolescentis	10%

Formulation E

Inulin	30%
FOS	15%
Immunoglobulin comp.	49.72%
L. acidophilus NCFM	2.5%
B. adolescentis	2.5%
Lactoperoxidase	0.03%
Sodium thiocyanate	0.05%
Lactoferrin	0.1%
Gluconic acid	0.1%

Formulation F

Inulin	40%
Pectin	9.98%
Immunoglobulin comp.	40%
B. adolescentis	10%
Lactoperoxidase	0.02%

Formulation G

Inulin	10%
FOS	10%
Pectin	10%
Guar Gum	10%
Immunoglobulin comp.	52.95%
L. acidophilus NCFM	7%
Potassium thiocyanate	0.05%

Formulation H

Inulin	50.9%
Immunoglobulin comp.	40%
B. adolescentis	9%
Lactoferrin	0.1%

Formulation I

Inulin	42%
Immunoglobulin comp.	40%
B. adolescentis	8%
Sodium gluconate	10%

Formulation J

Inulin	20%
FOS	20%
Pectin	4.44%
Guar Gum	1%
Immunoglobulin comp.	40%
B. adolescentis	10%
Lactoperoxidase	0.01%
Ammonium thiocyanate	0.05%
Sodium gluconate	4.5%

Formulation K

FOS	36%
Pectin	3.5%
Guar Gum	2.5%
Immunoglobulin comp.	42%
L. acidophilus NCFM	10%
Lactoferrin	0.01%
Gluconic acid	5.99%

Formulation L

Inulin	40%
Immunoglobulin comp.	40%
B. adolescentis	10%
Lactoperoxidase	0.0001%
Sodium thiocyanate	0.0001%
Lactoferrin	0.0001%
Gluconic acid	10%

Claims

I claim:

1. An immunoglobulin and fiber-containing composition comprising in percent by weight

(a) about 40 to about 60% of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins; and

(b) about 40 to about 60% of soluble dietary fiber, wherein said fiber is a member selected from the group consisting of inulin, fructo-oligosaccharides, pectin, guar gum, and mixtures thereof.

2. The composition of claim 1 further comprising about 0.1 to about 20% by weight of a beneficial human intestinal microorganism selected from the group consisting of lactobacilli and bifidobacteria.

3. The composition of claim 1 further comprising about 0.0001% to 0.0500% by weight of thiocyanate salt and about 0 to about 0.0300% by weight of lactoperoxidase.

4. The composition of claim 2 further comprising about 0.0001% to about 0.0500% by weight of thiocyanate salt and about 0 to about 0.0300% by weight of lactoperoxidase.

5. The composition of claim 4 comprising about 0.0001% to about 0.0300% by weight of lactoperoxidase.

6. The composition of claim 1 further comprising about 0.0001% to about 0.1000% of lactoferrin and about 0 to about 10% by weight of a member selected from the group consisting of gluconic acid, its nutritionally acceptable salts, and mixtures thereof.

7. The composition of claim 2 further comprising about 0.0001% to about 0.1000% of lactoferrin and about 0 to about 10% by weight of a member selected from the group consisting of gluconic acid, its nutritionally acceptable salts, and mixtures thereof.

8. The composition of claim 3 further comprising about 0.0001% to about 0.1000% of lactoferrin and about 0 to about 10% by weight of a member selected from the group consisting of gluconic acid, its nutritionally acceptable salts, and mixtures thereof.

9. The composition of claim 4 further comprising about 0.0001% to about 0.1000% of lactoferrin and about 0 to about 10% by weight of a member selected from the group consisting of gluconic acid, its nutritionally acceptable salts, and mixtures thereof.

10. The composition of claim 9 comprising about 0.1% to about 10% by weight of a member selected from the group consisting of gluconic acid, its nutritionally acceptable salts, and mixtures thereof.

11. The composition of claim 2 wherein said beneficial human intestinal microorganism is a member selected from the group consisting of *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum,* and *L. cellobiosus*.

12. The composition of claim 11 wherein said beneficial human intestinal microorganism is *Lactobacillus acidophilus*.

13. The composition of claim 12 wherein said *Lactobacillus acidophilus* is strain NCFM.

14. The composition of claim 2 wherein said beneficial human intestinal microorganism is a member selected from the group consisting of *Bifidobacterium adolescentis, B. infantis, B. longum, B. thermophilum,* and *B. bifidum.*

15. The composition of claim 14 wherein said beneficial human intestinal microorganism is *B. adolescentis.*

16. The composition of claim 1 wherein said immunoglobulin composition further comprises a carrier.

17. The composition of claim 16 wherein said carrier comprises at least one member selected from the group consisting of a carbohydrate and a lipid, wherein said carbohydrate is capable of being an energy source for a beneficial human intestinal microorganism and said lipid aids in reconstitution of said immunoglobulin composition.

18. The composition of claim 17 wherein said carbohydrate comprises maltodextrin and said lipid comprises lecithin.

19. The composition of claim 1 wherein said immunoglobulin composition is purified from a source selected from the group consisting of milk, milk products, and whey.

20. The composition of claim 19 wherein said source is bovine.

21. A bacteria and immunoglobulin-containing composition for promoting gastrointestinal health comprising

(a) an effective amount of a beneficial human intestinal microorganism; and

(b) an effective amount of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins.

22. The composition of claim 21 wherein said beneficial human intestinal microorganism is selected from the group consisting of lactobacilli and bifidobacteria.

23. The composition of claim 22 wherein the weight ratio of beneficial human intestinal microorganism to immunologically active immunoglobulins is in the range of about 20:1 to about 1:20.

24. The composition of claim 23 wherein weight ratio of beneficial human intestinal microorganism to immunologically active immunoglobulins is in the range of about 1:5 to about 10:1.

25. The composition of claim 24 wherein said beneficial human intestinal microorganism is a lactobacillus.

26. The composition of claim 25 wherein said lactobacillus is selected from the group consisting of *L. acidophilus, L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum, and L. cellobiosus*.

27. The composition of claim 26 wherein said lactobacillus is *Lactobacillus acidophilus*.

28. The composition of claim 27 wherein said *Lactobacillus acidophilus* is strain NCFM.

29. The composition of claim 24 wherein said beneficial human intestinal microorganism is a bifidobacterium.

30. The composition of claim 29 wherein said bifidobacterium is selected from the group consisting of *Bifidobacterium adolescentis, B. infantis, B. longum, B. thermophilum, and B. bifidum.*

31. The composition of claim 30 wherein said bifidobacterium is *B. adolescentis.*

32. The composition of claim 21 wherein said immunoglobulin composition further comprises a carrier.

33. The composition of claim 32 wherein said carrier comprises at least one member selected from the group consisting of a carbohydrate and a lipid, wherein said carbohydrate is capable of being an energy source for said beneficial human intestinal microorganism and said lipid aids in reconstitution of said immunoglobulin composition.

34. The composition of claim 35 wherein said carbohydrate comprises maltodextrin and said lipid comprises lecithin.

35. The composition of claim 21 wherein said immunoglobulin composition is purified from a source selected from the group consisting of milk, milk products, and whey.

36. The composition of claim 35 wherein said source is bovine.

37. A method of restoring and maintaining gastrointestinal health comprising the step of orally

administering a bacteria and immunoglobulin-containing composition comprising an effective amount of a beneficial human intestinal microorganism and an effective amount of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins.

38. The method of claim 37 wherein said beneficial human intestinal microorganism is a member selected from the group consisting of lactobacilli and bifidobacteria.

39. The method of claim 38 wherein the weight ratio of beneficial human intestinal microorganism to immunologically active immunoglobulins is in the range of about 20:1 to about 1:20.

40. The method of claim 39 wherein the weight ratio of beneficial human intestinal microorganism to immunologically active immunoglobulins is in the range of about 1:5 to about 10:1.

41. The method of claim 40 wherein said lactobacilli are selected from the group consisting of *L. acidophilus, L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum, and L. cellobiosus* and said bifidobacteria are selected from the group consisting of *Bifidobacterium adolescentis, B. infantis, B. longum, B. thermophilum, and B. bifidum.*

42. The method of claim 41 wherein said beneficial human intestinal microorganism is *Lactobacillus acidophilus.*

43. The method of claim 41 wherein said beneficial human intestinal microorganism is *B. adolescentis.*

44. The method of claim 41 wherein said immunoglobulin composition is purified from a source selected from the group consisting of bovine milk, milk products, and whey.

45. A method of restoring and maintaining gastrointestinal health comprising the step of orally administering an effective amount of an immunoglobulin and fiber-containing composition for promoting gastrointestinal health comprising in percent by weight

(a) about 40 to about 60% of an immunoglobulin composition comprising concentrated immunologically active immunoglobulins; and

(b) about 40 to about 60% of soluble dietary fiber, wherein said fiber is a member selected from the group consisting of inulin, fructo-oligosaccharides, pectin, guar gum, and mixtures thereof.

46. The method of claim 45 wherein said immunoglobulin and fiber-containing composition further comprises about 0.1 to about 20% by weight of a beneficial human intestinal microorganism selected from the group consisting of lactobacilli and bifidobacteria.

47. The method of claim 46 wherein said lactobacilli are selected from the group consisting of *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. fermentum, L. salivaroes, L. brevis, L. leichmannii, L. plantarum, and L. cellobiosus* and said bifidobacteria are selected from the group consisting of *Bifidobacterium adolescentis, B. infantis, B. longum, B. thermophilum, and B. bifidum*.

48. The method of claim 47 wherein said beneficial human intestinal microorganism is *Lactobacillus acidophilus*.

49. The method of claim 47 wherein said beneficial human intestinal microorganism is *B. adolescentis*.

50. The method of claim 45 wherein said immunoglobulin and fiber-containing composition further comprises about 0.0001% to 0.0500% by weight of thiocyanate salt and about 0 to about 0.0300% by weight of lactoperoxidase.

51. The method of claim 45 wherein said immunoglobulin and fiber-containing composition further comprises about 0.0001% to about 0.1000% of lactoferrin and about 0 to about 10% by weight of a member selected from the group consisting of gluconic acid, its nutritionally acceptable salts, and mixtures thereof.

52. The method of claim 45 wherein said immunoglobulin composition is purified from a source selected from the group consisting of bovine milk, milk products, and whey.

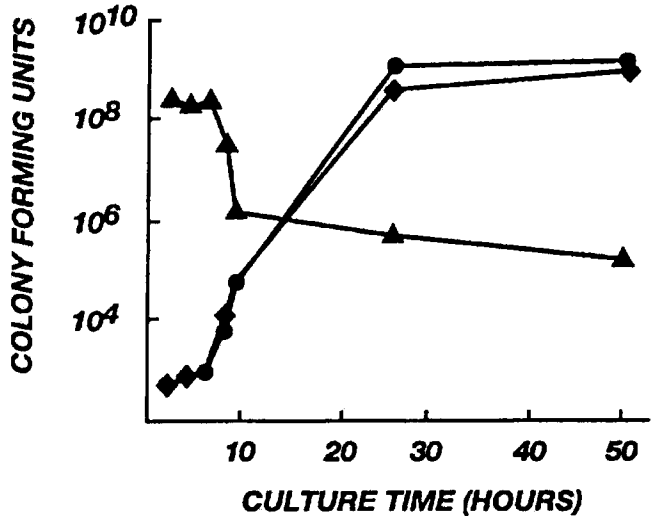

COLONY FORMING UNITS
10^{10}
10^{8}
10^{6}
10^{4}
10
20
30
40
50
CULTURE TIME (HOURS)

Fig. 1

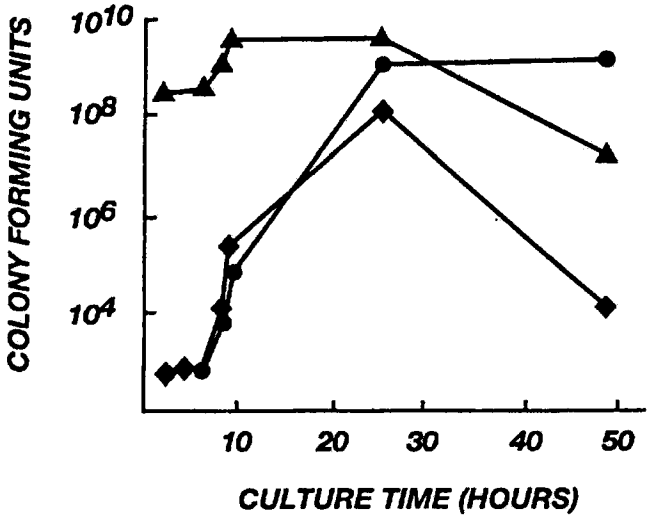
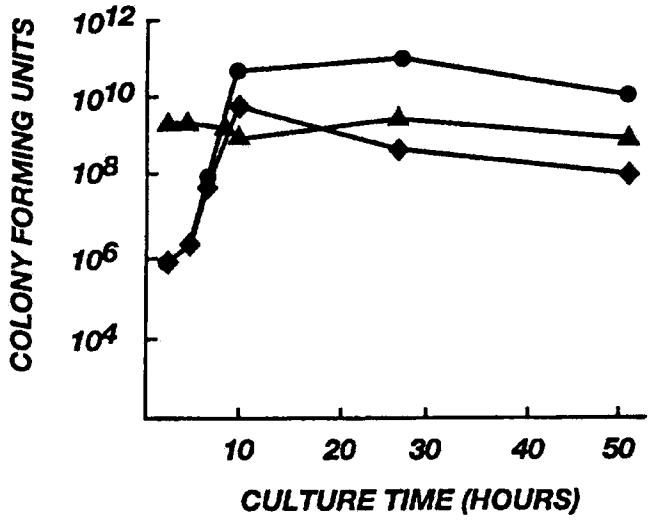

COLONY FORMING UNITS
10^{10}
10^{8}
10^{6}
10^{4}
10
20
30
40
50
CULTURE TIME (HOURS)

Fig. 2


COLONY FORMING UNITS
10^{10}
10^{8}
10^{6}
10^{4}
10
20
30
40
50
CULTURE TIME (HOURS)

Fig. 3


COLONY FORMING UNITS
10^{12}
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10^{8}
10^{6}
10^{4}
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30
40
50
CULTURE TIME (HOURS)

Fig. 4

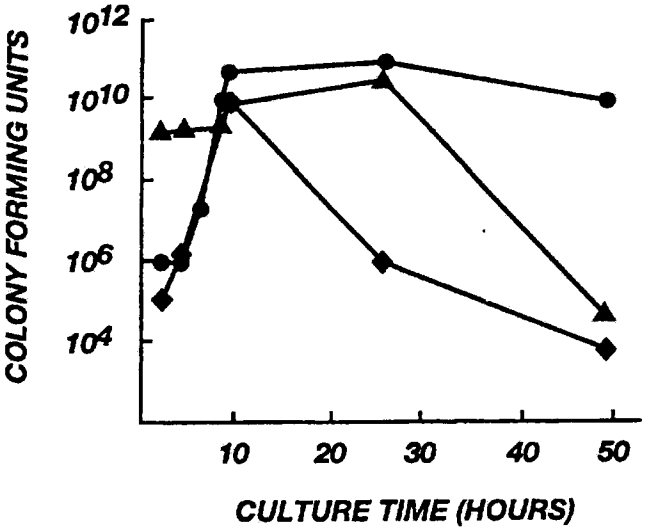
COLONY FORMING UNITS
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
10
20
30
40
50
CULTURE TIME (HOURS)

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13905

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 35/00, 35/20, 39/02, 39/07, 39/395, 39/40, 39/42, 47/00

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,816,252 (STOTT ET AL.) 28 MARCH 1989, see entire document.	1-52
A	US, A, 4,977,137 (NICHOLS ET AL.) 11 DECEMBER 1990, see entire document.	1-52
A	US, A, 5,240,909 (NITSCHE) 31 AUGUST 1993, see entire document.	1-52
A	Journal of Food Protection, Volume 40, Number 12, issued December 1977, Gilliland et al., "Antagonistic Action of *lactobacillus acidophilus* Toward Intestinal and Foodborne Pathogens in Associative cultures", pages 820-823, see entire document.	1-52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 JANUARY 1996

Date of mailing of the international search report
08 FEB 1996

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13905

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Dairy Science, Volume 73, Number 4, issued 1990, Gilliland et al., "Factors to Consider When Selecting a Culture of *Lactobacillus Acidophilus* as a Dietary Adjunct to Produce a Hypocholesterolemic Effect in Humans", pages 905-911, see entire document.	1-52
A	Annals of Internal Medicine, Volume 116, Number 5, issued 01 March 1992, Hilton et al., "Ingestion of Yogurt Containing *Lactobacillus Acidophilus* as Prophylaxis for Candidal Vaginitis", pages 353-357, see entire document.	1-52
A	New England Journal of Medicine, Volume 318, Number 19, issued 12 May 1988, Tacket et al., "Protection by Milk Immunoglobulin Concentrate Against Oral Challenge With Enterotoxigenic *Escherichia Coli*", pages 1240-1243, see entire document.	1-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13905

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/93.4, 93.45, 130.1, 143.1, 145.1, 147.1, 148.1, 149.1, 150.1, 151.1, 157.1, 158.1, 159.1, 161.1, 195.1, 234.1, 499, 500, 535, 809; 514/2, 439, 441, 445, 777, 867

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/93.4, 93.45, 130.1, 133.1, 135.1, 135.6, 141.1, 143.1, 145.1, 147.1, 148.1, 149.1, 150.1, 151.1, 157.1, 158.1, 159.1, 161.1, 195.1, 234.1, 499, 500, 535, 809; 514/2, 439, 441, 445, 484, 485, 777, 867